FINAL REPORT

19960430

ENHANCEMENT OF BRASSICA JUNCEA CANOLA BREEDING THROUGH MARKER-ASSISTED SELECTION

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Technical Report – April 3, 2002

Principal investigator J. Philip Raney (Van L. Ripley)

Title: Enhancement of *Brassica juncea* canola breeding through marker assisted selection.

A) Summary

There were two major objectives to this study. The first objective focussed on development of molecular markers for disease resistance and seed quality traits. Several *B.juncea* gene mapping populations were developed and molecular markers were identified for white rust resistance (race 2a and 2v) and seed glucosinolate content (allyl and butenyl glucosinolates). A mapping population was also produced to allow for mapping of oil content genes in *B.juncea*.

The second major objective was the development of improved germplasm for entry into the cooperative variety testing system. Germplasm was developed that had seed oil content equal to *B.napus* even under stressful field conditions. Germplasm was developed that had a fatty acid profile similar to *B.napus* with fatty acid profile of oleic acid 60-65%, linoleic acid 20% and linolenic acid of 10%. Germplasm was also produced that contained a reduced level of glucosinolates, with total glucosinolates of approximately 5 μmole/g of oil-free meal. The various improved components of germplasm were recombined through crossing and should allow for development of improved varieties for commercialization by 2006.

B) Technical report

Background and objectives

Brassica juncea has traditionally been grown as a condiment mustard crop and has been shown to have good adaptation to the hotter drier areas of western Canada. The acreage for all mustard production (brown, oriental and yellow) in western Canada has ranged from 280,000 acres to 800,000 acres from 1989-99 (Stats Canada 2000). *Brassica juncea* was originally chosen as a species for conversion to canola quality because it exhibits superior drought and heat tolerance, disease resistance and pod shatter resistance compared to *Brassica napus* (Woods et al. 1991, Burton et al. 1999). Mustard *B.juncea* was found to be higher yielding than either *B.napus* or *B.rapa* under droughty conditions (Woods et al., 1991). Initial results with the canola quality AAFC line J90-4316 indicated the canola quality germplasm has heat tolerance similar to *B.juncea* mustard (Angadi et al., 2000).

Present day *B.juncea* varieties are only suitable for the condiment trade as their seed oil contains high amounts of erucic acid and their meal contains high amounts of allyl glucosinolate (an anti-nutritive factor which limits of its value as an animal feed). *B.juncea* seed quality characteristics must be siginificantly improved to allow for establishment of this species as a canola species. Agronomic characteristics must also be improved. White rust, caused by *Albugo candida*, is an important disease of *B. juncea* in western Canada. Genetic resistance within cultivars is the most cost effective way to protect the crop from this disease. Mustard *B.juncea* cultivars are available, which are resistant to race 2a of white rust, but no native resistance to race 2v has been observed. Immunity to race 2v has been introgressed into *B. juncea* through an interspecific cross with *B. napus*. The greatest impact of our research will be the expansion of acreage under canola oil production through the development of canola quality *B. juncea* germplasm.

The availability of *B. juncea* as an oilseed crop will give producers in the drier growing regions of western Canada, the opportunity to include an oilseed crop in the monoculture, wheat-summer fallow rotation and diversify their production. In addition, *B. juncea* has the potential to replace the present canola species in the southern 1/3 of production areas, current species are lower yielding compared to our expectations of an adapted *B. juncea* variety. Taken together, there will be new acres planted for canola quality oils and a replacement of lower yielding species.

The results of this research will enhance Saskatchewan's agriculture industry in the area of special crops. The intention was to develop well adapted, high yielding, disease resistant, canola quality *B. juncea* germplasm and varieties for cultivation in Saskatchewan's drier environments. Conventional breeding practices and molecular marker-assisted selection will facilitate this goal.

The objectives of the study were to accelerate the development of high yielding, canola quality, oilseed *B. juncea* with improved white rust resistance and improved seed quality by increasing seed oil content, modifying the fatty acid profile and further reducing glucosinolate content. An additional objective is to develop a large suite of molecular markers for several traits for continued use in genetic enhancement of *B. juncea*. These improvements are essential to develop *B. juncea* as a sustainable, competitive, canola quality oilseed crop.

Project activities, achievements, papers written

Several mapping populations were developed to provide the genetic material for mapping the traits in the program and identifying molecular markers. Below is a summary of the crosses used in the genetic mapping and gene tagging studies.

QUALITY

High oil x low oil (high erucic acid background)

This population, from a cross of J90-2741 (high oil content oriental mustard line originating from Russian germplasm) by AC Vulcan (a relatively low oil content oriental mustard developed at AAFC-SRC), is an F_2 derived, single seed decent population consisting of approximately 250 lines at the F_5 plant stage. 199 F_6 rows were planted in 2000 together with the appropriate checks for phenotypic evaluation of oil content. The population has been advanced to the F_7 plant stage (approx. 200 lines). 190 F_8 lines were planted in a 2001 replicated field nursery for confirmation of oil contents observed in the 2000 nursery. Due to the large effects of environment on oil content, it was decided to place the 190 F_8 lines back in the field in 2002 for a confirmation of oil content. We feel the severe environmental conditions expressed in the field in 2001 may have resulted in the generation of poor phenotypic data. This phenotypic data will be used for the development of DNA markers linked to oil content and therefore it is vital correct oil content data is generated. We have performed a preliminary evaluation of the mapping population, however the final attempts at mapping this trait in this population can only be completed after the field season 2002.

Gene cloning of the components of acetylcholine carboxylase has been performed for both parents. The clones are available and ASA markers can be developed that will be used to tag the population to determine linkage of these markers to oil content once the final year of oil data is generated in the field. The candidate genes under investigation are derived from *B. napus* and *A. thaliana* and were identified from BLAST search of accession lines identified in the literature. Four major genes were initially pursued, beta-carboxylase, biotin carboxylase carrier protein, biotin carboxylase and alpha-carboxyltransferase. Efforts related to cloning of the biotin carboxylase carrier protein were discontinued due to the large number of published sequences for this gene.

High oil x low oil (low erucic acid background)

This population, from a cross of TO97-3422 by TO97-3421, is a F_2 derived, single seed decent population consisting of approximately 250 lines at the F_5 plant stage. TO97-3422 is a large-seeded zero erucic acid oriental mustard line. TO97-3421 is a high oil content, zero erucic acid line derived from a cross of J90-2741 by a zero erucic acid, low glucosinolate *B. juncea* line (J90-4253). 240 F_6 rows were planted in 2000 together with the appropriate checks for evaluation of oil content. The population has been advanced to the F_7 plant stage (approx. 200 lines). 188 F_8 lines were planted in a 2001 replicated field nursery for confirmation of oil contents observed in the 2000 nursery. Due to the large effects of environment on oil content, it was decided to place the 188 F_8 lines back in the field in 2002 for a confirmation of

oil content. We feel the severe environmental conditions expressed in the field in 2001 may have resulted in the generation of poor phenotypic data. This phenotypic data will be used for the development of DNA markers linked to oil content and therefore it is vital correct oil content data is generated. We have performed a preliminary evaluation of the mapping population, however final attempts at mapping this trait in this population can only be completed after the field season 2002.

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B. juncea 2733 x *B. juncea* 4317

A mapping population of 75 doubled haploid lines was developed and evaluated for two years in a replicated field nursery. The parental lines were chosen based on their diversity for a range of characteristics.

Juncea 2733 – high oil content, high glucosinolates, white rust resistant (race 2a)
Juncea 4317 – low oil content, low glucosinolates, white rust susceptible (race 2a)

Seed quality characteristics, including glucosinolate content has been determined. A genetic linkage map was created and several AFLP markers have been identified for the various traits.

Low linolenic, zero erucic acid interspecific *B. juncea* x (zero erucic acid *B. juncea*)

Previously, a zero erucic acid, low glucosinolate *B. juncea* line (J90-4253) was crossed with a low linolenic acid *B. napus* line (S86-69) received from the University of Manitoba. Interspecific F_1 plants were then backcrossed to *B. juncea*. For this project the interspecific BC_1F_1 plants were backcrossed to zero erucic acid *B. juncea* three times to produce BC_4F_1 seed. Finally, the BC_4F_1 generation was selfed and BC_4F_2 seed produced. At each generation, tissue from each of the plants was taken and tested for the presence of two DNA markers linked to low linolenic acid in *B. napus*. The BC_4F_2 seed was planted in a field nursery in 2000 and individual plants were selfed, tissue sampled, and individually harvested. The harvested seed was analyzed for fatty acid composition and the tissue taken examined for the presence of the *B. napus* DNA markers linked to low linolenic content. Selected BC_4F_1 lines were planted in 2001 to test the efficacy of molecular marker accelerated selection for

low linolenic acid in *B. juncea* and to develop a new source a low linolenic, zero erucic acid *B. juncea.*

DISEASE

Canola quality *B. juncea* (race 2v resistant) x AC Vulcan (race 2v susceptible)

[2V resistant/*3, 2V susceptible] We have BC_3F_1, BC_3F_2, BC_3F_3 plants all fully evaluated for race 2V reaction. Eight AFLP markers linked to resistance to white rust race 2v have been identified

AC Vulcan (race 2A resistant) x Lethbridge 22A and Forge (race 2a susceptible)

Reciprocal crosses were made and four populations developed. We have these populations as F_3 seed. Each population consists of approximately 200 F_2 lines. This seed was used for white rust resistance screening of F_3 plants for phenotypic evaluation of the F_2 lines. The AC Vulcan by Forge population was used to screen for AFLP markers using bulked segregant analysis. Twenty-two AFLP markers were mapped and found to be co-segregating with the 2A resistance locus. Three of the AFLP markers are extremely tightly linked to the resistance locus and a fourth marker is located close to the 2a resistance gene.

Results and Conclusions

Oil content:

The two populations in development will act as tools to map genetic loci controlling oil content in canola quality *B. juncea*. Our attempts at microspore culture and doubled haploid production in the early stages of the project were unsuccessful and had an impact on the timeline for development of the mapping populations. We have had great success with developing mapping populations from these crosses through single seed decent. These two populations were evaluated in two separate, replicated nurseries in 2000 and 2001 and oil content data generated. The range of oil contents in field trials in 2000, in the high erucic acid population ranged from AC Vulcan level (~41%) to nearly J90-2741 level (~52%). The range of oil contents observed in the low erucic acid population ranged ~39% to ~47%. The two replicates of each entry in the two nurseries were in close agreement with respect to oil content in most cases. In 2001, the material was tested in replicated nurseries and the range of oil contents in the high erucic population ranged from AC Vulcan (~37%) to J90-2741 level (~50%). The range of oil contents observed in the low erucic acid population ranged from ~35% to ~47%.

It is our opinion these populations must be evaluated for an additional year in the field due to the variation in levels of oil content observed in the field between the 2000 and 2001 field seasons (Table 1). The 2001 field season was characterized by a severe

drought which had a significant impact on the oil content levels observed. Identification of useful markers will only be achievable if we have an excellent understanding of the oil content phenotypes of these mapping populations. It is our goal to grow the mapping populations in the field in 2002 in order to obtain good oil content data on the mapping population to allow us to accurately complete the gene mapping and marker identification process.

Table 1. – Variation in oil content observed in mapping populations

Line ID	2000	2001	Range[a]
High erucic popn			
AC Vulcan	41%	37%	5%
J90-2741	52%	50%	2%
Low erucic popn			
97-3422	39%	35%	4%
97-3421	47%	47%	0%

a- Range indicates the range of variation seen for the lines listed in the table from the 2000 field season to the 2001 field season.

The impact of the drought of 2001 on oil content in the mapping population is evident in Table 1. The variation in oil content in the material could impact the assignment of mapping population individuals to the high or low oil content groups thereby limiting our ability to map this trait accurately.

In addition to the efforts on mapping population development, the breeding program has proceeded with the development of high oil *B.juncea*. Several high oil lines were identified in the nursery in 2000 and these were advanced to replicated trials in the 2001 field season. The high oil selections were tested at three locations and were found to have high yield and high oil potential (Table 2). The oil contents observed in this material were higher than the *B.napus* check. The high oil content was observed at multi-location trials in a severe drought year and therefore the results are a good indication of the potential of these lines.

Table 2. High oil *B.juncea* yield trials (3 locations)

Line ID	Rel. Yld[a]	Oil (%)	Glucosinolates(umoles)
Q2 (*B.napus*)	90%	43.7	14.7
T4672	132%	46.0	73.3[b]
T4676	143%	46.6	72.4
T4689	141%	45.7	60.1

- a-Rel. Yld. - % relative to canola quality *B.juncea* check
- b-zero allyl glucosinolate line

Acetylchöline carboxylase (ACCase) was selected as the putative gene associated with oil content variation. ACCase plays a regulatory role in fatty acid biosynthesis, which occurs in plastids. In the cytosol of plant cells, ACCase provides malonyl-CoA for fatty acid elongation, flavonoid formation and several other metabolic pathways. The multi sub-unit, also named prokaryotic type ACCase, consists of three dissociable components comprising a biotin carboxyl carrier protein sub-unit, a biotin carboxylase subunit and a carboxyltransferase complex consisted of non-identical alpha and beta sub-units. ACCase may be a limiting enzyme in lipid synthesis in rapeseed since its activity is reduced prior to full accumulation of lipids.

Table 3. ACCase genes identified in the literature

Gene	Accession #	Length (bp)	Source
1. beta-carboxyltransferase (accD)	Z50868	1668	*B.napus*
2. Biotin carboxyl carrier protein(accB)	X90727 X90728 X90729 X90730 X90731 X90732		*B.napus*
3. Biotin carboxylase (acc)	U90879	1985	*A. thaliana*
4. alpha-carboxyltransferase (accA)	AF056969	2821	*A. thaliana*

Cloning of putative ACCase genes of the four parent lines used in these two populations was completed. ACCase is comprised of four components and it appears each component is present in *B. juncea* as multiple copies, therefore a large number of gene clones had to be made. Five ACCase genes were cloned, covering the genes 1, 3 and 4, as listed in Table 3. The cloned genes have been compared to the published sequences in the literature. One cloned gene, identified as primer combination 9596 exhibited 98% similarity to the published sequence for beta-carboxlytransferase (accD) in *B. napus*. The original plan was to identify the point mutations in the cloned genes so that PCR based markers could be developed for use by the breeders. We feel it would be more appropriate to compare the sequence information we have generated with microsatellite and *Arabidopsis* sequence information to identify markers linked to the sequences we have identified. We have a significant amount of in-house data at the Saskatoon Research Centre related to microsatellites in *B.juncea* and therefore there is a high probability linked markers could be identified. Markers developed through this approach should also have a higher transferability between different breeding lines.

Glucosinolates:

There was cytological instability observed in the canola quality *B. juncea* parental lines that were originally proposed for experiments concerning glucosinolates. As a result, we have been able to use an older, pre-existing DH population that segregated quite well for glucosinolate levels and glucosinolate composition. The population was used to first construct a genetic map using AFLP markers. We have placed approximately 300 markers onto linkage groups. Field evaluation data was collected over two field seasons. Several AFLP markers were identified in this mapping population for glucosinolate content (Table 4).

Table 4. Identification of linked markers for glucosinolate content in the juncea 4317x2733 DH mapping population

Component	Markers identified	Linkage group[a]
Allyl glucosinolates	X0914H	2
	X0613C	2
	X0902D	2
	X0204O	8
	X0314E	8
Butenyl glucosinolates	X0604G	2
	X0314O	2
	X1016B	2
	X0915F	11

a- linkage group number represents our linkage group number as assigned during AFLP based map construction- does not relate to published linkage groups at this point for *B.juncea*

Analysis of our mapping population has identified several potentially useful AFLP markers that can now be converted to PCR based markers for confirmation of their utility and use by the breeding programs. We have identified what appears to be a major region located on our linkage group 2, containing genes involved in allyl and butenyl glucosinolate content in *B.juncea*. We now plan to integrate the information generated from this project with the *B.juncea* microsatellite map being generated by Dr. Derek Lydiate's group at the Saskatoon Research Centre. Integration of our markers to the microsatellite map will allow us to identify markers tightly linked to the genes controlling allyl and butenyl glucosinolate levels in *B.juncea*. The goal of the microsatellite mapping initiative is to develop a molecular map with molecular markers at equal intervals along each linkage group at a distance of less then 0.2 centimorgans. By aligning our map with the microsatellite map, we will have the potential to identify markers very tightly linked to the glucosinolate genes.

During the course of the mapping population development efforts, our breeding efforts to develop low glucosinolate material have proceeded. In the 2000 field season we were successful in identifying low glucosinolate material in the nursery

(Table 5). This material was extremely low in total glucosinoates, having both low allyl and low butenyl glucosinolate contents.

Table 5. Field evaluation in 2000 season of low glucosinolate *B.juncea* breeding lines

Line ID	Allyl	Butenyl	Total Alkenyl	Total Indole	Total Gluc.[a]
6765	0.0	0.8	1.1	3.1	4.4
6769	0.0	1.2	1.4	3.5	4.9
6796	0.1	3.2	3.6	3.6	7.4
6863	0.0	1.7	1.9	2.8	4.9
6864	0.0	1.5	1.7	2.8	4.8
6865	0.1	0.7	0.9	3.9	4.9
6866	0.0	2.3	2.7	3.9	6.7
6868	0.3	1.1	1.9	3.4	5.7

- a- all glucosinolate levels expressed as µmoles/gram of oil free seed

The material identified in the 2000 field season as being low glucosinolate material was tested in a replicated trial in the field in 2001. Only one location could be planted of this trial due to low seed volumes however, results from 2001 confirmed the low glucosinolate trait in this material (Table 6). The low glucosinolate material was also exhibiting good yield potential and had oil content level similar to the *B.napus* check AC Excel.

Table 6. Replicated yield trial evaluation of low glucosinolate *B.juncea* breeding lines.

Line ID	Allyl	But	Alk.	Ind.	Tot. Gluc.[a]	Oil (%)	Rel.Yld(%)[b]
6765	0.0	2.6	2.9	3.9	6.9	44.2	127
6769	0.7	1.9	2.9	3.5	6.7	44.7	120
6796	0.1	4.1	5.1	3.4	8.9	43.9	110
6863	0.0	3.0	3.5	3.6	7.2	44.4	115
6864	0.2	3.1	3.7	3.8	7.7	43.9	113
6865	2.2	4.2	7.0	3.5	11.1	44.8	118
6866	0.1	1.9	2.1	3.3	5.6	43.7	119
6868	0.0	2.1	2.7	3.1	5.9	39.1	111
AC Excel	0.1	4.6	14.7	4.7	21.0	45.4	100
J90-2741	73.9	3.3	77.5	1.9	82.1	50.0	118
AC Vulcan	105.0	3.7	108.8	0.6	111.9	39.0	117
J90-4316	0.8	14.7	18.0	3.6	22.7	41.5	125

- a- all glucosinolate values expressed in µmoles/gram of oil free meal
- b- yield expressed as a percentage relative to the *B.napus* check AC Excel

The replicated trial with the low glucosinolate material indicated the low glucosinolate trait in these lines was consistent between years. The low glucosinolate material also exhibited good oil content and yield potential. The best material was recombined with our high oil and improved fatty acid material to allow for production of our first varieties.

A manuscript on the aneuploidy encountered in the original mapping population parental material was developed and reported. Funding from the AFIF grant was acknowledged in this report.

Title: Low glucosinolate *Brassica juncea* breeding line revealed to be nullisomic. 2001. B.F. Cheng, G. Seguin-Swartz, D.J. Somers and G. Rakow. Genome 44: 738-741

It is our intention to write a manuscript related to the mapping efforts using 4317 x 2733 and to submit it to a peer reviewed journal.

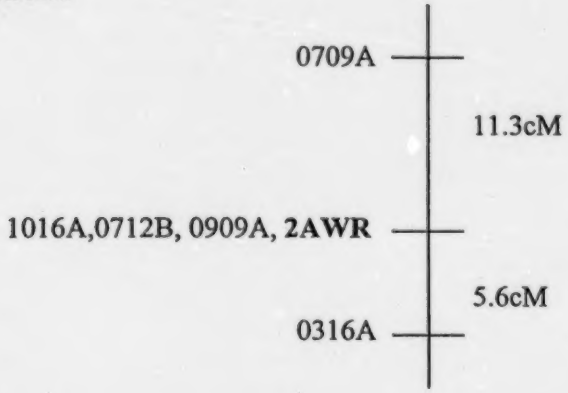
White rust (A. candida) race 2v resistance:

Previously, a zero erucic acid, low glucosinolate *B. juncea* line (J90-4253) was crossed with a low linolenic acid *B. napus* line (S86-69) received from the University of Manitoba. Interspecific F_1 plants were then backcrossed to *B. juncea* repeatedly with reselection for resistance to white rust race 2v. Cytological instability in some canola quality lines proposed for use in tagging white rust resistance genes in *B. juncea* was observed. However, we were able to make use of a BC_3 oriental mustard population (backcrossing to AC Vulcan) segregating for race 2v resistance developed by Gerhard Rakow. Working together with Roger Rimmer, the BC_3F_2 and BC_3F_3 plants were assessed for race 2v resistance and the results showed a simple Mendelian inheritance indicating a single dominant gene. AFLP technology was used along with bulked segregant analysis to identify eight *B. napus* fragments of DNA introgressed into *B. juncea* which contain a gene(s) conferring race 2v resistance in *B. juncea*. These markers are now being converted to more breeder friendly markers and will be implemented in the canola quality *B. juncea* breeding program.

White rust (A. candida) race 2a resistance:

Reciprocal crosses were made and four populations developed using the parental material AC Vulcan (2a resistant) x Lethbridge 22A (2a susceptible) or AC Vulcan x Forge (2a susceptible). One mapping population, AC Vulcan x Forge was analyzed for linkage of AFLP markers to the 2a resistance gene. Bulked segregant analysis was performed to identify putative AFLP markers linked to the trait. Ninety-six AFLP markers were identified for further analysis and were tested against the components of the bulks. Of the 96 markers evaluated, 22 were linked to the trait, with 5 markers being tightly linked at the resistance locus or flanking the locus (Figure 1).

Figure 1. AFLP molecular markers linked to 2a white rust Resistance in *B.juncea*



The markers that have been mapped to the 2a white rust resistance locus are being converted to PCR based markers for use in the *B.juncea* breeding program.

A manuscript on the development of markers derived from *B. napus* linked to white rust (2v) immunity in *B. juncea* was written to report these results. AFIF funding was acknowledged in this report.

Title: *Brassica napus* DNA markers linked to white rust immunity in *Brassica juncea*
Authors: Somers, D.J., Rakow, G. and Rimmer, S.R.
Published in Theoretical & Applied Genetics, Feb, 2002

Low linolenic acid, zero erucic acid B. juncea:

BC_4F_2 seed, segregating for the presence of two *B. napus* derived markers linked with low linolenic acid content, was planted in a nursery in 2000 together with checks. Individual plants in this nursery were bag selfed, tissue sampled for DNA marker analysis and individually harvested. The fatty acid composition of the seed oil of these plants was determined and compared with the presence or absence of the low linolenic acid *B. napus*. The presence of two *B. napus* low linolenic acid markers was correlated with decreased synthesis of linolenic acid from linoleic acid in the early backcross generations. The correlation of the low linolenic acid markers with the low linolenic acid trait appeared to breakdown in the most advanced backcross generation i.e. BC5 (see Table 7). Backcross five generation, individual plants with low linolenic acid level did not all contain the low linolenic acid markers. It appears as though there was recombination between the low linolenic acid markers and the locus controlling the low linolenic acid trait during the backcross procedure. Many backcrosses of the material to *B.juncea* were required since the original source of the low linolenic acid trait was a *B.napus* low linolenic line S86-69. Unfortunately, the large number of backcrosses also provided a greater opportunity for recombination between the markers and the gene controlling the low linolenic acid trait.

Our approach of analyzing the segregating germplasm through ½ seed analysis at most of the backcross generations allowed us to obtain *B.juncea* material with lower linolenic acid content. The ½ seed analysis approach was fairly labor intensive, however it was a prudent approach given the fact we appeared to have lost the markers during the backcrossing due to recombination.

Table 7. Development of low linolenic *B.juncea* through marker assisted breeding

			BC5F3 ½ Seed analysis[a]		
Line ID	559[b]	FAD3[b]	C18:1	C18:2	C18:3
4909-2	+	-	62.2	20.0	7.6
4910-1	+	-	62.5	20.8	8.5
4910-3	+	-	62.6	21.8	7.7
4912-1	-	-	64.7	21.4	4.3
4912-3	-	-	61.9	23.5	5.7
4912-4	-	-	61.4	24.2	5.1

- a- ½ seed analysis performed on greenhouse grown seed
- b- low linolenic acid markers developed in *B.napus* low linolenic line S89-69

Indications for further developments

All the required genetic elements (high oil content, zero erucic acid, increased oleic acid content/decreased linolenic acid content, very low glucosinolate content with no allyl glucosinolate present) to make *B. juncea* a canola crop competitive with *B. napus* in terms seed quality are available in various lines. The challenge is to integrate all these elements together in one line of *B. juncea*. The development of DNA markers for these seed quality traits will greatly assist in this work. There is excellent potential to further refine the markers identified in this study through alignment of our information with the microsatellite map being developed for *B.juncea*. The improvement of our markers will be pursued in the future.

Information on performance indicators

The project should still be able to meet the timeline of development of the first canola quality *B.juncea* variety by 2006 with improved oil content, lower glucosinolates and improved disease resistance. The project experienced some delays due to the problems with DH production for mapping population development and the abnormal chromosome situations in the low glucosinolate material.

Increased market share/sales/production/profits/exports

The first canola quality *B.juncea* varieties will be grown in western Canada in 2002. The first two varieties, Arid and Amulet, were developed by Saskatchewan Wheat Pool and these two varieties will help to establish the crop as a canola crop in Canada. It is expected germplasm and molecular markers developed in this AFIF project will allow

AAFC to quickly develop canola quality *B.juncea* varieties to help establish this new crop and increase its market share in the industry.

Increased membership/employment/patents

None

Identification and dissemination of new technological opportunities

When the first improved canola quality *B.juncea* variety is developed and registered, the variety will be advertised for tender for increase and distribution. Interested parties will have the opportunity to bid for marketing and distribution rights.

Increased knowledge of the human resource base

Mr. Brad Hope was a technician on this project for a period of time and has since gone to the University of Saskatchewan, Biochemistry Dept. to pursue a Master of Science degree. His interest and skill level in plant molecular biology was enhanced through the skills he acquired while working on the project.

Diversification and impact on production/producers/marketplace

This will become known when *B. juncea* becomes available to producers as another type of canola. It is expected the first varieties from SWP will be introduced to the marketplace in 2002.

Personnel

The following personnel were paid from project #96000430 funds for 2001/2002.

Name	Time	Salary (actual)
J. Helston (Tech.)	12 months	$43,898
T. Forge (Tech)	2 months	$5,802

Equipment

No purchases this past year.

Project Photos

None

Innovation Fund Acknowledgment

Somers, D.J., Rakow, G., Raney, P., Prabhu, V., Séguin-Swartz, G., Rimmer, R., Gugel, R., Lydiate, D. and Sharpe, A. (1999) Developing marker-assisted breeding for quality and disease resistance traits in Brassica oilseeds. 10th International Rapeseed Congress, Canberra, Australia (9/99) (invited).

Project public information materials

A manuscript on the investigation of chromosomal abnormalities in the low glucosinolate *B.juncea* material initially used for mapping glucosinolate genes was published. The funding by AFIF for this work was acknowledged in the paper.

Title: Low glucosinolate *Brassica juncea* breeding line revealed to be nullisomic. 2001. B.F. Cheng, G. Seguin-Swartz, D.J. Somers and G. Rakow. Genome 44: 738-741.

A manuscript on the development of markers derived from *B. napus* linked to white rust immunity in *B. juncea* has been published. The funding by AFIF for this work was acknowledged in this manuscript.

Title: *Brassica napus* DNA markers linked to white rust immunity in *Brassica juncea*
Authors: Somers, D.J., Rakow, G. and Rimmer, S.R.
Theoretical & Applied Genetics. Feb. 2002 (online publication initially)

Deliverables for evaluation

"Development of an adapted, high yielding, canola quality *B.juncea* oilseed to replace canola species in the southern 1/3 of the growing area and add two million acres to the western Canadian canola production base."

Significant progress towards development of germplasm helping to establish this new crop in the marketplace has been achieved by this project. Germplasm with improved oil content, improved fatty acid profile, lower glucosinolate content and good disease resistance was produced by the project. Replicated yield trials were performed with material and they indicated stable material was produced. In addition to development of material with improved quality and disease characteristics, high yielding material was identified. The improved germplasm exhibited a high level of drought and heat tolerance in the 2001 field season, out competing *B.napus* and mustard *B.juncea* material. The germplasm developed in this project was recombined and this material will be placed in the field in 2002. We anticipate it will lead to a variety combining all of the improvements. If recombinants are identified in the field in 2002 containing all of the necessary attributes, these lines could go into replicated trials in 2003, coop trials in 2004 and 2005 and be introduced to the marketplace in 2006.

Notes on yearly goals

Year 1 – "establish a molecular mapping lab to assist in the development of *B.juncea*"

This goal was accomplished. A molecular lab capable of plant molecular techniques such as RAPD, AFLP, PCR, gene cloning and DNA sequencing was established and operating by the end of the first year. Since mapping populations had already been developed in the *B.napus* program, the new techniques could be applied immediately to some projects in this species. We focussed on the development of markers for traits in *B.napus* we felt would be useful for accomplishing our goals in *B.juncea,* such as low linolenic acid and blackleg resistance.

Year 2,3 – " during the first two years of the project molecular markers will be developed for the various traits ...Doubled haploid populations will be developed that are true breeding for the various traits, and these will be used in crosses to combine traits under selection. Marker assisted selection will be utilized to identify recombinant genotypes."

Some aspects of this goal were delayed by problems encountered in the production of doubled haploid mapping populations, by the presence of abnormal chromosome numbers in some of the material used as parents and by abnormal field conditions during evaluation of the material in the field. The specific aspects affected were the oil content gene mapping and the glucosinolate mapping project. Markers were developed on schedule for white rust, linolenic acid level and blackleg resistance. As soon as we became aware the production of doubled haploids was going to be a limiting factor in the program, population development based on single seed decent (SSD) was initiated. We developed populations to the F7 and F8 generations, however this took additional time then would have been required for the DH approach. Three generations of selfing can be accomplished in the greenhouse in a given year therefore we initiated the SSD populations in 1998, which meant we did not have the mapping populations for the field until the 2000 field season. Initially we felt that we could evaluate the material in the field in 2000 and 2001 field season however, in 2001, a severe drought resulted in variation in oil content levels and it was therefore decided to proceed with a third year of evaluation in 2002. We returned to an earlier *B.juncea* glucosinolate mapping population when it became apparent the planned material contained aneuploidy (abnormal chromosome number).

Year 4,5 "During the last two years, the material developed will be field tested, first in nurseries, followed by multi-location yield testing of selected lines to identify superior, high yielding, disease resistant, high quality lines for entry into cooperative canola tests at the end of the project"

This goal was accomplished. We successfully proved development of low glucosinolate, high oil and improved fatty acid profile in the field. The material was tested in nurseries in the field in 2000 and the best material went to multi-location trials in 2001 where sufficient seed was available. As superior material was identified from the project, the

best material was recombined and re-selected. We have provided information in the document indicating we have produced germplasm improved for the specific traits we were pursuing and with substantial yield potential. The best, recombined material from this project has the potential to be advanced to the cooperative testing system.